Plasma and tissue concentrations of α-tocopherol during vitamin E depletion in sheep

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(*Received 11 September 1991 – Accepted 12 March 1992*)

To determine the relationship between plasma and tissue α-tocopherol concentrations during vitamin E depletion, weaned lambs were placed on a vitamin E-deficient diet for 0, 1, 2, 4, 8 and 12 weeks. α-Tocopherol was measured in plasma, erythrocytes, liver, adrenal, adipose tissue, three different skeletal muscles and heart muscle. The α-tocopherol concentration in plasma fell at the same rate as the α-tocopherol concentration in skeletal muscles, heart muscle, adrenal and adipose tissue. The α-tocopherol concentration in liver and erythrocytes fell at a faster rate than that of plasma and all muscle tissues. There were significant correlations between α-tocopherol concentration in plasma and α-tocopherol concentrations in all the tissues measured. Different skeletal muscles had significantly different concentrations of α-tocopherol which may relate to their differing susceptibility to nutritional myopathy. The increase in malondialdehyde in oxidatively-stressed muscle tissue and the correlation with α-tocopherol concentration in most muscle tissues indicated that the muscles had reduced antioxidant capacity *in vitro* as a result of vitamin E depletion. It was concluded that during vitamin E depletion in sheep α-tocopherol concentration in plasma was a good index of vitamin E status under the experimental conditions employed.

α-Tocopherol: Vitamin E: Nutritional myopathy: Sheep

Vitamin E deficiency can be an important factor in the development of nutritional myopathy in ruminants (Steele *et al.* 1980; Rice & McMurray, 1986). Measurements of α-tocopherol in plasma are used as an index of vitamin E status in ruminants which assumes that there is a direct relationship between the plasma and the tissue concentrations of α-tocopherol (McMurray *et al.* 1983). However, there have been conflicting reports as to the validity of using plasma α-tocopherol as an index of vitamin E status in a variety of species.

Wiss *et al.* (1962) in studies in chickens found that plasma α-tocopherol was linearly related to log liver α-tocopherol only when intakes of α-tocopherol were high. Pudelkiewicz & Mary (1969) found a linear relationship between log liver α-tocopherol and log plasma α-tocopherol and suggested that plasma α-tocopherol did provide an index of the vitamin E status of the animal when dietary intake was kept constant to stabilize plasma and liver α-tocopherol. Hidiroglou & Charmley (1990), in an experiment in sheep fed on graded dietary levels of vitamin E, concluded that there was no significant correlation between plasma and liver α-tocopherol concentration and that only liver α-tocopherol concentration was a good indicator of vitamin E status.

Studies in humans have led to the suggestion that plasma α-tocopherol would be a better indicator of vitamin E status if related as a ratio to plasma lipids (Horwitt *et al.* 1972; Thurnham *et al.* 1986). The present study tested the validity of using plasma α-tocopherol, or plasma α-tocopherol:lipid ratio as an indicator of vitamin E status in sheep that were

depleted in vitamin E. Since muscle tissue is the tissue most affected by vitamin E deficiency in ruminants, it was also considered important to determine the effect of vitamin E depletion on α-tocopherol concentration in this tissue and its relationship to the α-tocopherol concentration in plasma.

MATERIALS AND METHODS

Animals and diet

Merino wethers, 4 months of age and weighing between 19·8 and 35·5 kg, were acquired off green pasture. At weaning at 3 months of age they had received Se and Co intraruminal pellets (Permasel Se pellets and Permaco Co pellets respectively; Coopers, Pitman-Moore Australia Ltd, North Ryde, NSW). On arrival at the animal house they were drenched with an anthelminthic, then acclimatized to the new environment and adapted onto a barley–chaff ration (800 g barley, 200 g chaff) over a 2-week period. They were supplemented with 15 mg α-tocopherol/d over this period. The sheep were kept in a single pen and provided with adequate trough space and *ad lib.* water.

On day 0 of the experiment thirty-four sheep were randomly allocated from live-weight strata into six groups. Eight sheep were allocated to the control group and these were bled and killed on day 0 for necropsy. The other five experimental groups contained either five or six sheep and were designated to be fed on a vitamin E-deficient ration for 1, 2, 4, 8 or 12 weeks before being killed for necropsy. The vitamin E-deficient ration was fed from day 0 and consisted of 800 g barley and 200 g chaff, plus 20 g ground limestone per sheep per d. The barley and chaff had been treated with NaOH by the method of Rice *et al.* (1985) to deplete them of vitamin E. The α-tocopherol concentration in the ration was less than 1 mg/kg (barley 0·6 mg/kg, chaff 2·5 mg/kg).

Measurements

Body weights were measured weekly. Before each group was killed for necropsy, blood samples were collected from that group for the measurement of erythrocyte glutathione peroxidase (*EC* 1.11.1.9) and plasma α-tocopherol, cholesterol and triacylglycerol. Plasma creatine kinase (*EC* 2.7.3.2) was measured at 0, 8 and 12 weeks to monitor for muscle damage. At necropsy the following samples were collected for determination of their α-tocopherol concentration: dorsal lobe of liver, adrenal glands, interventricular septum of the heart, perirenal adipose tissue and three skeletal muscles (*tensor fascia lata*, *vastus lateralis* and *vastus intermedius*). Portions of *vastus lateralis* were collected into formalin (100 ml/l) for subsequent microscopic examination of haematoxylin and eosin-stained paraffin sections. At 0 and 12 weeks samples of the same muscles and of heart were collected for determination of malondialdehyde (MDA).

Analytical techniques

α-Tocopherol was measured in feed, erythrocytes, tissues and plasma using a HPLC method with fluorometric detection (McMurray & Blanchflower, 1979). Tissues and erythrocytes were saponified by the method of Bieri *et al.* (1961). Erythrocyte glutathione peroxidase was measured using an automated modification of the method of Paynter *et al.* (1985). Creatine kinase, cholesterol and triacylglycerol were measured by automated methods at 37° using Roche Unikits CK NAC, Cholesterol PAP and Triglycerides INT respectively (Roche Diagnostic Systems, Hoffman La Roche Inc., New Jersey, USA). MDA was produced in muscle homogenates oxidatively stressed by incubating them with 10 μmol $FeCl_3$/l and 100 μmol ascorbic acid/l by the method of Jackson *et al.* (1983). MDA was measured as the MDA-thiobarbituric acid (MDA–TBA) adduct by HPLC with

fluorometric detection using a modification of the method of Wong *et al.* (1987). The emission wavelength was 500 nm and the excitation wavelength 532 nm. The flow-rate was 1·5 ml/min. The Se concentration of muscle tissues was measured on a dry-weight basis by the method of Gabbedy *et al.* (1977).

Statistical analysis

Logarithms were taken of all α-tocopherol concentrations and MDA concentrations in order to stabilize variance. Linear regressions between α-tocopherol and time were fitted and their slopes were compared using *t* tests. Correlations were calculated between the plasma α-tocopherol concentration, or the plasma α-tocopherol:cholesterol plus triacylglycerol concentration value and the α-tocopherol concentration in tissues. Correlations were calculated between the α-tocopherol concentration and the MDA production. All other comparisons were by analysis of variance.

RESULTS

Concentrations of α-tocopherol in different tissues with time are shown in Fig. 1 (*a–c*), together with the fitted regression lines. Regression coefficients are shown in Table 1. In all tissues the α-tocopherol concentration fell rapidly on the depletion diet. The rate of depletion as determined by the slope on the regression line was not significantly different in plasma, adrenal, adipose tissue or any of the muscle tissues. The rate of depletion of α-tocopherol from liver was significantly faster than the rate from plasma and muscle tissue but was not significantly different from the rate of depletion from adrenal or adipose tissue.

The correlation between plasma α-tocopherol and tissue α-tocopherol concentrations is shown in Table 2. The correlations were not improved by expressing plasma α-tocopherol relative to cholesterol and triacylglycerol concentrations. All correlations were highly significant ($P = 0{\cdot}0001$).

The slopes of the regressions of log α-tocopherol concentration *v.* time were the same for all the skeletal muscles so the mean α-tocopherol concentrations of all the samples of each skeletal muscle were compared using analysis of variance including time and muscle effects. The mean α-tocopherol levels were significantly different in the three different skeletal muscles ($P = 0{\cdot}0001$). The re-transformed means (mg/kg) with 95% confidence limits were: *tensor fascia lata* 1·09 (0·92–1·26), *vastus lateralis* 1·49 (1·23–1·75), *vastus intermedius* 2·18 (1·81–2·55).

The MDA content of muscle after stimulation of oxidation was significantly higher in *vastus intermedius* ($P = 0{\cdot}011$), *vastus lateralis* ($P = 0{\cdot}001$) and heart muscle ($P = 0{\cdot}001$) at week 12 compared with controls (week 0). The re-transformed means of MDA content of the muscles are shown in Table 3. There was a significant negative correlation between the α-tocopherol concentration and the MDA produced in homogenates of heart muscle (r 0·84, $P = 0{\cdot}003$), *vastus intermedius* (r 0·61, $P = 0{\cdot}027$) and *vastus lateralis* (r 0·68, $P = 0{\cdot}001$) but not in *tensor fascia lata* (r 0·47, $P = 0{\cdot}099$).

No sheep developed clinical myopathy, although there was evidence from microscopic examination of the *vastus lateralis* of mild sub-clinical myopathy in two sheep (one at week 2 and one at week 8) during the period of vitamin E depletion. Plasma creatine kinase remained within the reference range (< 250 U/l). Plasma cholesterol and triacylglycerol concentrations were significantly lower in sheep after 1 week on the diet ($P = 0{\cdot}001$). Plasma cholesterol concentration fell from a mean of 1·56 (SE 0·09) mmol/l to a mean of 1·01 (SE 0·04) mmol/l and triacylglycerol from 0·32 (SE 0·03) mmol/l to 0·15 (SE 0·02) mmol/l, but there was no significant difference from week 1 to the rest of the period on the diet. Glutathione peroxidase levels ranged from 366 to 672 U/g haemoglobin. The Se level in the muscles ranged from 0·37 to 0·77 mg/kg dry weight.

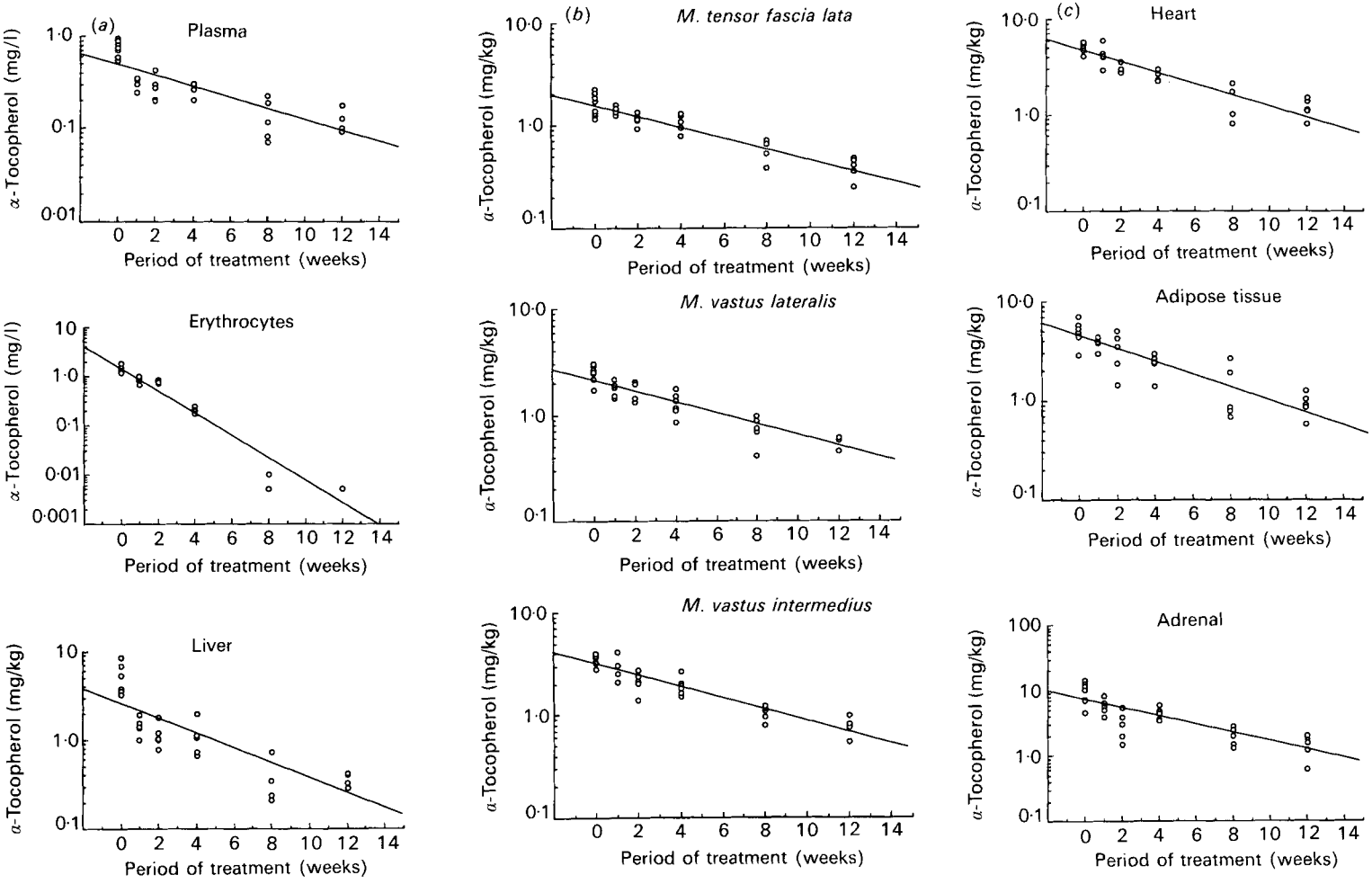

Fig. 1. α-Tocopherol concentration in plasma and tissues of sheep fed on a vitamin E-deficient diet for 0, 1, 2, 4, 8 and 12 weeks. (*a*) Plasma, erythrocytes and liver, (*b*) skeletal muscles: *tensor fascia lata*, *vastus lateralis* and *vastus intermedius* and (*c*) heart, adipose tissue and adrenal from vitamin E-deficient sheep (for details of treatment, see p. 226).

Table 1. *Regression coefficients for linear relationships between the α-tocopherol concentration and duration of depletion for vitamin E-depleted sheep**

(Mean values with their standard errors)

Tissue	Intercept† Mean	SE	Slope Mean	SE	R^2
Plasma	−0·3216	0·0455	−0·05784[a]*	0·00779	0·64
Erythrocytes	0·1595	0·0588	−0·22610[c]	0·01010	0·94
Liver	0·4063	0·0572	−0·08365[b]	0·00979	0·69
Adrenal	0·8365	0·0473	−0·06393[ab]	0·00809	0·66
Adipose	0·6355	0·0372	−0·06202[ab]	0·00637	0·74
Heart	0·6580	0·0257	−0·05520[a]	0·00440	0·83
M. tensor fascia lata	0·1946	0·0257	−0·05286[a]	0·00358	0·86
M. vastus lateralis	0·3317	0·0244	−0·05324[a]	0·00417	0·83
M. vastus intermedius	0·4989	0·0221	−0·05467[a]	0·00379	0·86

[a,b,c] Means with unlike superscript letters were significantly different ($P < 0·05$).
* For details of treatment, see p. 226.
† Standard error of the estimated coefficient.

Table 2. *Correlations between log plasma α-tocopherol concentrations and log tissue α-tocopherol concentrations for vitamin E-depleted sheep**

Erythrocytes	0·804
Liver	0·970
Adrenal	0·863
Adipose	0·843
Heart	0·906
M. vastus lateralis	0·847
M. vastus intermedius	0·841
M. tensor fascia lata	0·819

All correlations are significant at $P = 0·0001$.
* For details of treatment, see p. 226.

Table 3. *Re-transformed means of malondialdehyde content (nmol/g protein) of muscles from vitamin E-depleted sheep†*

Muscle	Week 0 (*n* 8)	Week 12 (*n* 5)
Vastus intermedius	1972	3419*
Vastus lateralis	546	1498*
Tensor fascia lata	1672	2067
Heart	607	2154*

* Means were significantly different from those for week 0 ($P < 0·05$).
† For details of treatment, see p. 226.

DISCUSSION

The results show that there was a significant correlation between plasma and tissue levels of α-tocopherol during depletion of vitamin E in sheep. The rate of depletion in plasma was the same as in all other tissues except liver and erythrocytes. The faster rate of depletion

in the α-tocopherol concentration in the liver may relate to its role in regulating other tissue pools (Hidiroglou, 1987), although this does not prevent the plasma levels from falling rapidly. Bieri (1982) and Machlin *et al.* (1979), in experiments in rats and guinea-pigs respectively, reported a biphasic pattern in the depletion of α-tocopherol from plasma and liver. A similar pattern of depletion occurred in the present experiment but was not as apparent with the logarithmic transformation of α-tocopherol concentration. In contrast to their results, the α-tocopherol level in adipose tissue depleted at the same rate as that of other tissues.

Thurnham *et al.* (1986) found that the α-tocopherol: triacylglycerol plus cholesterol ratio was almost as powerful as the α-tocopherol: total lipid ratio. In the present study expressing the plasma α-tocopherol relative to triacylglycerol plus cholesterol concentration did not improve the correlation with tissue α-tocopherol. This may not be the case in a situation where lipid levels are pathologically lowered or elevated.

Allen *et al.* (1986) suggested that nutritional myopathy in weaner sheep may be a type II muscle fibre disease. Salviati *et al.* (1980) after injection of rabbits with radiolabelled α-tocopherol found higher levels associated with homogenates and subcellular fractions of muscles with predominantly type I fibres than those with type II fibres. They also found greater incorporation of α-tocopherol into sarcoplasmic reticulum membranes of type I muscles *in vitro* and suggested that this may explain the greater susceptibility of type II muscles to myopathy due to vitamin E deficiency in rabbits. Of the three skeletal muscles examined in the present study the *vastus intermedius* had the highest α-tocopherol concentration. This muscle is composed mainly of type I fibres (White *et al.* 1978; Richards *et al.* 1988). The *vastus lateralis*, which is a mixture of type I and type II fibres but predominantly type II fibres (White *et al.* 1978), and the *tensor fascia lata*, which is composed mainly of type II fibres (Richards *et al.* 1988), had lower concentrations of α-tocopherol than the type I fibre muscle. Thus, higher concentrations of protective α-tocopherol may be the reason lesions are less common in muscles with predominantly type I fibres in sheep.

The other indicator of susceptibility to myopathy is the antioxidant capacity of the muscle. The MDA concentration after increasing the oxidative stress with Fe and ascorbate is an indicator of the antioxidant capacity of the muscle (Jackson *et al.* 1983). The MDA concentration was increased at the end of the depletion period compared with controls in the *vastus intermedius*, the *vastus lateralis* and heart muscle. This increase in MDA could be related to an increase in substrate (as polyunsaturated fatty acids) or to a decrease in the antioxidant capacity of the tissue (as vitamin E). Rice *et al.* (1986) found that the NaOH treatment of the barley *per se* caused an increase in linoleic acid in the phospholipids of skeletal muscle tissue of calves. Linoleic acid is relatively resistant to peroxidation and is not believed to cause production of MDA (Kornbrust & Mavis, 1980). Buttriss & Diplock (1988) also found that in the organelles of vitamin E-deficient rats linoleic acid in the phospholipids behaved like the monounsaturated fatty acids in that it was not destroyed to any degree by peroxidation. The significant negative correlation between MDA concentration and α-tocopherol concentration in the individual tissues in the present study suggests that the increase in peroxidation with oxidative stressing was in fact related to the deficiency in α-tocopherol rather than any change in substrate in the tissues.

In the present study sheep were so depleted of vitamin E that their status could be defined as deficient. This represents the situation that can lead to the development of nutritional myopathy. The diagnosis of a vitamin E deficiency is important in determining the aetiology of the myopathy and the most appropriate treatment. In the field the rate of depletion of body stores of vitamin E will vary with a number of factors but primarily with the vitamin E content of the feed. Liver α-tocopherol concentrations in weaned lambs with myopathy

(Steele *et al.* 1981) can deplete over approximately the same time period to levels as low as in the present experimental study. Even if overall depletion rates are different it is unlikely that the direct relationship between plasma and tissue levels will alter. The present study has demonstrated that the plasma α-tocopherol concentration does provide a good index of the vitamin E status of sheep experimentally depleted of α-tocopherol. This suggests that plasma α-tocopherol may be useful as a diagnostic indicator of vitamin E deficiency.

The authors thank Mr M. Hare and Mr K. Reynolds for maintenance of the animals. This study was supported by research funds from the Australian Wool Corporation Wool Research and Development Council.

REFERENCES

Allen, J. G., Steele, P., Masters, H. G. & D'Antuono, M. F. (1986). A study of nutritional myopathy in weaner sheep. *Australian Veterinary Journal* **63**, 8–13.

Bieri, J. G. (1972). Kinetics of tissue α-tocopherol depletion and repletion. *Annals of the New York Academy of Sciences* **203**, 181–191.

Bieri, J. G., Pollard, C. J., Prange, I. & Dam, H. (1961). The determination of α-tocopherol in animal tissues by column chromatography. *Acta Chemica Scandinavica* **15**, 783–790.

Buttriss, J. L. & Diplock, A. T. (1988). The α-tocopherol and phospholipid fatty acid content of rat liver subcellular membranes in vitamin E and selenium deficiency. *Biochimica et Biophysica Acta* **963**, 61–69.

Gabbedy, B. J., Masters, H. & Boddington, E. B. (1977). White muscle disease of sheep and associated tissue selenium levels in Western Australia. *Australian Veterinary Journal* **53**, 482–484.

Hidiroglou, M. (1987). Vitamin E levels in sheep tissues at various times after a single oral administration of D-α-tocopherol acetate. *International Journal of Vitamin and Nutrition Research* **57**, 381–384.

Hidiroglou, M. & Charmley, E. (1990). Response of plasma and tissue D-α-tocopherol in sheep to graded dietary levels of DL-α-tocopherol acetate. *Research in Veterinary Science* **49**, 122–124.

Horwitt, M. K., Harvey, C. C., Dahm, C. H. & Searcy, M. T. (1972). Relationship between tocopherol and serum lipid levels for determination of nutritional adequacy. *Annals of the New York Academy of Sciences* **203**, 223–236.

Jackson, M. J., Jones, D. A. & Edwards, R. H. T. (1983). Vitamin E and skeletal muscle. In *Biology of Vitamin E. Ciba Foundation Symposium* no. 101, pp. 224–234 [R. Porter and J. Whelan, editors]. London: Pitman.

Kornbrust, D. J. & Mavis, R. D. (1980). Relative susceptibility of microsomes from lung, heart, liver, kidney, brain and testes to lipid peroxidation: correlation with vitamin E content. *Lipids* **15**, 315–322.

Machlin, L. J., Keating, J., Nelson, J., Brin, M., Filipski, R. & Miller, O. N. (1979). Availability of adipose tissue tocopherol in the guinea pig. *Journal of Nutrition* **109**, 105–109.

McMurray, C. H. & Blanchflower, W. J. (1979). Application of high performance liquid chromatographic fluorescence method for the rapid determination of α-tocopherol in the plasma of cattle and pigs and its comparison with direct fluorescence and high performance liquid chromatography ultraviolet detection methods. *Journal of Chromatography* **178**, 525–531.

McMurray, C. H., Rice, D. A. & Kennedy, S. (1983). Nutritional myopathy in cattle: from a clinical problem to experimental models for studying selenium, vitamin E and polyunsaturated fatty acid interactions. In *Trace Elements in Animal Production and Veterinary Practice. Occasional Publication* no. 7, pp. 61–73 [N. F. Suttle, R. G. Gunn, W. M. Allen, K. A. Linklater and G. Wiener, editors]. Edinburgh: British Society of Animal Production.

Paynter, D. I., Halpin, C. G. & Caple, I. W. (1985). Measurement of blood glutathione peroxidase activity for assessment of selenium nutrition in livestock. *Australian Standard Diagnostic Techniques for Animal Diseases* no. 45. *Australian Agricultural Council Standing Committee on Agriculture*. Melbourne: CSIRO.

Pudelkiewicz, W. J. & Mary, N. (1967). Some relationships between plasma, liver and excreta tocopherol in chicks fed graded levels of alpha-tocopherol acetate *Journal of Nutrition* **98**, 303–306.

Rice, D. A., Blanchflower, W. J. & McMurray, C. H. (1985). The effects of moisture, propionic acid, sodium hydroxide and anaerobis on the stability of vitamin E in stored barley. *Journal of Agricultural Science, Cambridge* **105**, 15–19.

Rice, D. A., Kennedy, S., McMurray, C. H. & Blanchflower, W. J. (1986). Differences in tissue concentrations of n-3 and n-6 fatty acids in vitamin E and selenium deficient cattle. *Proceedings of the 6th International Conference on Production Disease in Farm Animals*, pp. 229–232. Belfast: Veterinary Research Laboratory.

Rice, D. A. & McMurray, C. H. (1986). Use of sodium hydroxide treated selenium deficient barley to induce vitamin E and selenium deficiency in yearling cattle. *Veterinary Record* **118**, 173–176.

Richards, R. B., Passmore, I. K. & Dempsey, E. F. (1988). Skeletal muscle pathology in ovine congenital progressive muscular dystrophy. *Acta Neuropathologica* **77**, 95–99.

Salviati, G., Betto, R., Margeth, A., Novello, F. & Bonetti, E. (1980). Differential binding of vitamin E to sarcoplasmic reticulum from fast and slow muscles of the rabbit. *Experientia* **36**, 1140–1141.

Steele, P., McKenzie, D. P., Skirrow, S., Peet, R. L. & Doncon, G. (1981). Selenium and tocopherol treatment of ovine weaner nutritional myopathy *Trace Element Metabolism in Man and Animals* (*TEMA*-4), pp. 210–213 [J. McHowell, J. M. Gawthorne and C. L. White, editors]. Canberra: Australian Academy of Science.

Steele, P., Peet, R. L., Skirrow, S., Hopkinson, W. & Masters, H. G. (1980). Low alpha-tocopherol levels in livers of weaner sheep with nutritional myopathy. *Australian Veterinary Journal* **56**, 529–532.

Thurnham, D. I., Davies, J. A., Crump, B. J., Situnayake, R. D. & Davis, M. (1986). The use of different lipids to express serum tocopherol:lipid ratios for the measurement of vitamin E status. *Annals of Clinical Biochemistry* **23**, 514–520.

White, N. A., McGavin, M. D. & Smith, J. E. (1978). Age related changes in percentage of fiber types and mean fiber diameters of the ovine quadriceps muscles. *American Journal of Veterinary Research* **39**, 1297–1302.

Wiss, O., Bunnel, R. H. & Gloor, W. (1962). Absorption and distribution of vitamin E in the tissues. *Vitamins and Hormones* **20**, 441–445.

Wong, S. H. Y., Knight, J. A., Hopfer, S. M., Zaharia, O., Leach, C. N. Jr. & Sunderman, F. W. Jr. (1987). Lipoperoxides in plasma as measured by liquid-chromatographic separation of malondialdehyde-thiobarbituric acid adduct. *Clinical Chemistry* **33**, 214–220.

Printed in Great Britain